

# *Environmental Effects of Dredging Technical Notes*



## **Chronic Sublethal Sediment Bioassays for the Regulatory Evaluation of Marine and Estuarine Dredged Material: Proceedings of a Workshop**

### **Purpose**

This note summarizes the proceedings of a workshop held April 3-5, 1990, at the US Army Engineer Waterways Experiment Station, Vicksburg, MS. The purpose of the workshop was to solicit input from technical experts regarding techniques for evaluating the chronic sublethal effects of sediments on aquatic biota. This input will be used to help direct subsequent research and development by the US Army Corps of Engineers.

### **Background**

The US Army Corps of Engineers uses an effects-based approach for the regulatory evaluation of dredged material. Bioassays are conducted to determine the toxicity of sediments and the bioaccumulation potential of sediment-associated contaminants. Survival of appropriate sensitive test species is used to measure acute sediment toxicity. This endpoint is quantal; that is, the test species either lived or died. Interpretation, therefore, is relatively straightforward.

Animals exposed to sediment normally accumulate contaminants at a slow rate compared to animals exposed to contaminants in aqueous solutions. Thus, sediment exposures connote chronic chemical exposures. Such chronic, low-level exposures are often not fatal but may elicit one or more subtle sublethal responses in the organism. These biological responses are designed to be adaptive. However, the chemical exposure may be of sufficient magnitude or duration that the

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organism's survival potential is impaired. Methods to accurately determine the chronic sublethal effects of sediment are not well developed. Moreover, the ability to discern adaptive from maladaptive sublethal responses (that is, interpret test results) is even more rudimentary.

The US Army Corps of Engineers has statutory authority for evaluating chronic impacts of dredged material. Regulations implementing Section 103 of the Marine Protection, Research and Sanctuaries Act (PL 92-532) state that, "[M]aterial shall be deemed environmentally acceptable for ocean dumping only when . . . no significant undesirable effects will occur due either to chronic toxicity or to bioaccumulation . . ." Likewise, regulations implementing Section 404(b)(1) of the Clean Water Act (PL 92-500) state that, "[T]he permitting authority shall determine in writing the potential short-term or long-term effects of a proposed discharge of dredged or fill material on the physical, chemical and biological components of the aquatic environment . . ."

In response to this statutory authority and technical need, a new research work unit, Chronic Sublethal Effects of Contaminated Dredged Material on Aquatic Organisms, was initiated within the Long-Term Effects of Dredging Operations (LEDO) Program.

## **Additional Information or Questions**

Contact one of the authors, Dr. Tom Dillon, (601) 634-3922, Ms. Alfreda Gibson, (601) 634-4027, Dr. David Moore, (601) 634-2910, or the manager of the Environmental Effects of Dredging Programs, Dr. Robert M. Engler, (601) 634-3624.

## **Introduction**

Workshop participants were welcomed by COL Fulton, Commander and Director of the US Army Engineer Waterways Experiment Station (WES), and Dr. Tom Dillon, Workshop Chairman. Each participant was asked to briefly introduce himself and describe his technical background. All participants are recognized technical experts representing private industry, academia, and the Federal government (Table 1). A number of scientists from the US Environmental Protection Agency R&D laboratories (Narragansett, RI; Gulf Breeze, FL; and Newport, OR) were invited but were unable to attend.

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Table 1. Workshop Participants

Mr. Steven Bay	Southern California Coastal Water Research Project, Long Beach, CA
Dr. Scott Carr	US Fish & Wildlife Service, Corpus Christi, TX
Dr. Ed Casillas	NOAA/National Marine Fisheries Service, Seattle, WA
Dr. Ted DeWitt	Oregon State University, Newport, OR
Dr. Jay Means	Louisiana State University, Baton Rouge, LA
Dr. David Moore	University of South Carolina, Columbia, SC
Dr. Frank Reilly	ASCI Corp., McLean, VA
Dr. John Scott	SAIC Inc., Narragansett, RI
Dr. Jack Word	Battelle Marine Research Laboratory, Sequim, WA
Dr. Tom Dillon, Chairman	US Army Engineer Waterways Experiment Station (WES)
Ms. Joan Clarke	WES
Dr. Robert Engler	WES
Ms. Freda Gibson	WES
Dr. Tom Fredette	New England Division, Waltham, MA
Mr. John Wakeman	Seattle District, Seattle, WA

Dr. Robert Engler presented the national perspective on the Corps' regulatory program for dredged material testing (Engler and others 1988). He explained the "effects-based" approach and the tiered testing protocol. He noted that while efforts are underway within the R&D community to develop chronic sublethal sediment tests, there are no generally accepted standard bioassays appropriate for the routine regulatory evaluation of dredged material. For that reason, use of these tests in a regulatory environment is restricted to special situations, for example, when biologically important bioaccumulation is observed with no concomitant acute toxicity.

Mr. John Wakeman, US Army Engineer District, Seattle, indicated that in the Pacific Northwest there is a broad-based constituency calling for the use of chronic sublethal testing in regulatory programs. In response, the Puget Sound Water Quality Authority has directed the Washington Department of Ecology to develop and eventually incorporate chronic sublethal tests into a variety of regulatory and monitoring activities. They have identified a 20-day growth bioassay with the marine benthic polychaete, *Neanthes arenaceodentata*, as a desirable chronic sublethal test (Johns and Ginn 1990). This procedure is also being considered by the Puget Sound Dredged Disposal Analysis (PSDDA) program for evaluating dredged material. The Corps' technical opinion is that more development is needed before this test is ready for use in a regulatory context.

Dr. Tom Fredette, US Army Engineer Division, New England (NED), described the New England Division's extensive monitoring program at aquatic relocation sites for dredged material. Although historical monitoring goes back to the 1930s, formal testing under the Disposal Area Monitoring System (DAMOS) did not begin until the 1970s. NED recently modified their testing protocol to include a 10-day bioassay with the amphipod *Ampelisca abdita* and 28-day bioassays with *Macoma balthica* and *Nereis virens*. Contaminant bioaccumulation potential will be assessed with the latter two species. Dr. Fredette indicated they would use a chronic sublethal sediment bioassay if it was technically sound, fully developed, ecologically relevant, and could be used in lieu of current testing procedures.

Dr. Frank Reilly summarized the results of a related workshop recently held at WES. The subject of that workshop was genotoxicity. This is a specific category of sublethal test that is being evaluated at WES under a separate but parallel effort. To avoid duplication, genotoxic endpoints were not addressed in any great detail during the current workshop. Details of the genotoxicity workshop will be reported in a future Environmental Effects of Dredging Technical Note (Reilly and others in preparation).

Dr. Dillon outlined objectives of the workshop and charged the participants with providing their best specific technical guidance. To initiate discussions, a hypothetical regulatory situation was described to the attendees (a permit action for marina dredging). They were asked to recommend a chronic sublethal sediment bioassay. They were specifically requested to address each of the workshop objectives by indicating how long the test would be run, what sublethal endpoint(s) would be monitored, what test species would be used, and how the test would be interpreted in terms of issuing or denying the permit. Response to this mock regulatory exercise is summarized in Table 2 and formed the basis for subsequent discussions at the workshop.

### **Workshop Objective 1: How Long is "Chronic"?**

Current regulatory bioassays for evaluating the toxicity of dredged material may last up to 10 days. Since these are typically referred to as "acute" toxicity tests, one could infer that "chronic" tests are longer than 10 days. But how much longer? From the participants' response (Table 2), 3-6 weeks appears to be an appropriate timeframe. However, it was not possible to reach consensus on a time-specific criterion for the term "chronic" because the lifespan of aquatic animals can range from a few days to many months. The participants felt a specific time for chronic would be too restrictive.

Instead, two important characteristics of chronic sediment exposure were identified. The exposure should include a substantial portion of the life cycle or number of life stages and allow sufficient time so that contaminant steady-state is approached in the tissues. Although the terms "substantial" and "sufficient" are qualitative, they provide necessary flexibility since duration of life cycles and time to steady-state vary tremendously. This concept of chronic requires that one

demonstrate, or at least convincingly argue, that the two constraints have been met.

Table 2. Characteristics of Chronic Sublethal Sediment Bioassays Suggested by the Workshop Participants

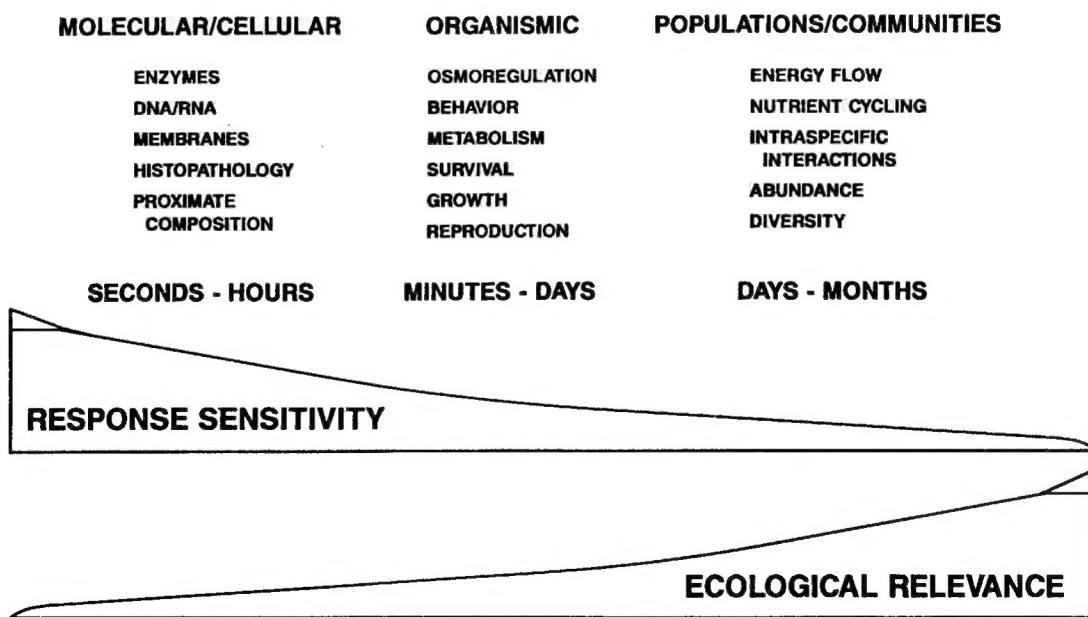
<u>Test Duration, days (unless otherwise noted)</u>	<u>Sublethal Endpoint(s)*</u>	<u>Test Organism</u>
28	reproduction	amphipod
14	challenge	any species
28	growth, reproduction	amphipod
30	growth, reproduction	amphipod
10-40	growth, reproduction	amphipod
20-60	growth, reproduction	amphipod
28-60	growth, reproduction	amphipod
20	growth	polychaete
28	growth	polychaete
30	growth	polychaete
20-60	growth, reproduction	polychaete
21-120	growth, reproduction	polychaete
45-60	growth, genotoxic biomarkers	polychaete
45-60	growth, genotoxic biomarkers	adult bivalve
not specified	genotoxic biomarkers	adult bivalve
2-4	development, genotoxic aberrations	larval bivalve
2-4	development, genotoxic aberrations	larval sea urchin
15 min.	bioluminescence inhibition	Microtox®

\* All participants suggested survival also be reported.

## **Workshop Objective 2: Identify Appropriate Sublethal Endpoints**

Only one endpoint in acute lethality tests is possible, percent survival. In contrast, the number of sublethal endpoints is almost infinite. They can be arranged according to three levels of biological organization: biochemical/cellular, organismic or whole animal, and populations/communities (Figure 1). The ultimate goal of most environmental protection programs is the maintenance of healthy viable populations and communities. Consequently, effects on populations and communities have the highest ecological relevance and societal importance. However, response sensitivity at this level of biological organization is generally low and predictive methods are not well developed. At the other extreme, biochemical/cellular endpoints may be quite sensitive but their ecological relevance is often unclear. Evaluating the response of individual whole organisms represents a judicious compromise between response sensitivity and ecological relevance. This approach, referred to as the surrogate toxicological approach, is used by many regulatory agencies (including the Corps) in evaluating contaminant-related perturbations.

During workshop discussions, the attendees upheld the proposition that the ability of an organism (or population of organisms) to reproduce and remain viable is of paramount ecological importance. Desirable sublethal endpoints should assess this capability. The participants identified two sublethal responses almost exclusively which fulfill this requirement: growth and reproduction (Table 2).



**Figure 1. Levels of Biological Organization**

Growth is a measure of change in mass or dimension. It can be expressed as a rate function or in absolute terms. The participants indicated that measuring growth in individuals was superior to estimating individual growth from survival and biomass. Measuring biomass alone was deemed unacceptable. Because growth and reproduction both represent competing demands on a usually limited energy source, the participants felt it was extremely important to distinguish between somatic growth and gametic growth (that is gametogenesis) in both measurement and interpretation.

The maintenance of viable populations is dependent on two factors, reproduction and survival. Sublethal measures of reproductive success would therefore seem to have greater intrinsic value than observations of growth. However, the costs associated with evaluating reproductive success are usually much greater (that is, longer and more complex experiments) than those that just measure growth. Also, most participants felt that if growth was adversely affected, reproduction would likely be affected to some degree. The question then arose, "Is growth an acceptable surrogate measure for reproduction?" After much discussion, the following conclusions were agreed upon:

1. The most desirable sublethal measure is reproduction, especially if expressed in terms of population viability.
2. If one measures only growth, the relationship between growth and reproduction *must* be thoroughly researched and quantitatively expressed.
3. The biological importance of any growth diminution must be interpreted in light of the relationship between growth and reproduction.
4. Enhanced growth due to experimental treatment is possible. If that result is observed, then treatment effects on reproductive success *must* be evaluated.
5. Measures of growth must distinguish between somatic and gametic growth.

### **Workshop Objective 3: Identify Appropriate Test Species**

Before discussing individual species, the participants were asked to formulate a prioritized list of criteria for use in selecting appropriate test species. The ranked criteria are shown below.

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- 1st - Intimate contact with sediment
- 2nd - Amenable to testing\*

\* Includes the following:

- Unaffected by nontreatment influences (for example, sediment grain size).
- Readily available from lab cultures or field collections.
- Reasonable cost.
- Appropriate endpoints (that is, growth or reproduction).
- Defined precision in control and reference response.
- Logistically feasible.

3rd - Ecological relevance and sensitivity (tied)

4th - Economic importance

Prioritization of the first two selection criteria (intimate contact with sediment and amenable to testing) were clear choices among the participants. Ranking the next two criteria (ecological relevance and sensitivity) was more equivocal. As a result, they have been rated equally. The participants felt that the last criterion (economic importance) should be considered only when all other factors are equal. However, this criterion may become very important if, for example, a commercially important species is at clear and demonstrated risk.

Amphipods and polychaetes were the participants' main choices for test species (Table 2). Among the amphipods, chronic sublethal effects methodologies are most developed for *Ampelisca abdita* (Scott and Redmond 1989). *Ampelisca* are estuarine infaunal tube dwellers that occur from the intertidal environment down to about 60 m. They are surficial detrital feeders but must be fed an algal diet in the laboratory. The life cycle can be completed in 28-30 days at 20-25°C. Most tests have been conducted with field-collected animals. Current research is focused on the development of culture techniques, appropriate feeding rations, and improvement of the survival of lab-reared young. Also under development are a partial life-cycle test protocol (<20-day test) and a demographic population model.

Another recommended amphipod was *Grandidierella japonica*. Its distribution, life cycle, and feeding habits are similar to *Ampelisca abdita*. However, it is a much larger amphipod and constructs a membranous tube that is not as substantial as that of *Ampelisca*. It can be maintained in the laboratory on ground fish flake food. While *Grandidierella japonica* appears to be a good candidate species, test method development for this species lags behind that of *Ampelisca abdita*.

Word and others (1989) have reported that the sensitivities of *Ampelisca abdita* and *Grandidierella japonica* were similar to another amphipod, *Rhepoxynius abronius* in static 10-day bioassays. The latter species has been used extensively in acute toxicity sediment bioassays. However, it appears to be inappropriate for chronic sublethal testing. *Rhepoxynius* is an annual species. Gravid females are available only once a year. Attempts to culture this species have been unsuccessful. Attempts to culture other amphipod species (for example, *Lepidactylus* sp., *Leptocheirus* sp., and *Eohaustorius* sp.) as a prelude to chronic sublethal sediment bioassays are underway.

Development of a chronic sublethal sediment test method with polychaetes has focused on one species, *Neanthes arenaceodentata*. This polychaete is unique among the family Nereidae in that it has a nonplanktonic larval stage. Development is direct and a full life cycle can be completed in about 120 days. Cultures are easily maintained in the laboratory and organisms are widely available. *Neanthes arenaceodentata* has been used to evaluate the chronic sublethal effects (that is, growth and reproduction) of a variety of contaminants (Reish 1985). In addition, a population dynamics model has been constructed for this species (Pesch and others 1987).

Mr. John Wakeman indicated early in the workshop that growth in this species is being used as a sublethal sediment test in the Puget Sound area. However, important technical questions remain before this test can be used in the regulatory environment. Two research issues that need to be resolved are the relationship between growth and reproduction and the effects of important nontreatment factors (for example, ammonia, grain size, and feeding).

Dr. Ed Casillas described another polychaete species, *Armandia brevis*, which has recently been examined as a chronic sublethal test species. This polychaete is an obligate deposit feeder found in the shallow intertidal zone of Puget Sound and is available nine months of the year. The majority of the worm's adult growth occurs during the 20-day growth test. Current research is focused on culture techniques and the influence of nontreatment factors.

A life-cycle test using another marine worm, *Dinophilus gyrociliatus*, was described by Dr. Scott Carr. This species attains a maximum length of 1 mm and has a life cycle of 10 days at 20°C. *Dinophilus* is easy to culture and its short life cycle allows reproductive endpoints to be evaluated quickly. Because of its small size, it is not possible to test sediments directly with this organism. Instead pore water is extracted from sediments and used in the bioassay (Carr, Williams, and Fragata 1989). To obtain pore water, sediments are pressurized in a Teflon container with compressed air and the resultant effluent is filtered and frozen. Prior to testing, samples are thawed and adjusted to standard water quality conditions. The advantage of this procedure is that samples can presumably be frozen for extended periods of time and nontreatment effects such as grain size are avoided.

However, some participants were concerned that other more serious artifacts may be introduced using this procedure. For example, adsorption of contaminants during the extraction process or to the walls of the test vessel may occur. Also, the mass of contaminants may be depleted during static chronic exposures. Dr. Word presented evidence which suggests that pore water characteristics and subsequent toxicity are dramatically affected by the physical disturbance of sediment. Dr. Jay Means went on to explain that contaminant bioavailability is highly dependent on the type and amount of colloidal material in the pore water (Sigleo and Means 1990). This material is most certainly altered in pore-water extractions. Furthermore, contaminants that are tightly bound to sediment particles under hypoxic reducing conditions become mobile and available for biouptake when aerobic oxidizing conditions are imposed (Folsom and others 1988). For these reasons, it is highly unlikely that extraction and subsequent testing of pore water even remotely simulates sediment exposure. Pore-water exposure is also contrary to the highest priority criterion for organism selection identified by the workshop participants, namely, intimate contact with the sediment.

Additional candidate test species other than amphipods and polychaetes were identified by the attendees. Mr. Steven Bay described sediment bioassays he has conducted using the white sea urchin, *Lytechinus pictus*. This epibenthic urchin is a surface deposit feeder found at depths of 1-100 m off the southern California coast. It can be spawned and raised in the laboratory. Sublethal endpoints are

growth (test diameter, wet weight, and gonad weight) and behavior (sediment preference and activity). The gonads can also be excised and analyzed for contaminant bioaccumulation. Mr. Bay also clearly demonstrated how interstitial hydrogen sulfide concentrations may explain diminished growth in sediment-exposed sea urchins.

Dr. Casillas explained how, on a national basis, the National Oceanic and Atmospheric Administration and the National Marine Fisheries Service had become interested in chronic sublethal testing as a result of their Status and Trends program. His Seattle office became involved in sublethal sediment bioassays at the request of the local Corps District. Dr. Casillas identified three candidate species: harpacticoid copepod, *Tigriopus californicus* (Misitano and Schiewe 1990), juvenile sand dollar, *Dendraster excentricus*, and larval surf smelt, *Hypomesus pretiosus* (Casillas and others 1989). The copepod bioassay may last 3-10 weeks and uses reproduction as the sublethal endpoint. The echinoderm and fish assays both measure growth and last 28 days and 4 days, respectively.

## **Workshop Objective 4: Develop Interpretive Guidance**

Much of the workshop was required to effectively address the first three objectives. Consequently, only a limited amount of time was available for developing interpretive guidance for chronic sublethal sediment testing. The participants were able to draw a distinction between statistically significant differences and biologically important differences. The former is arbitrary to the extent that test results are dependent on the level of significance selected by the investigator (for example,  $P < 0.05$  versus  $P < 0.01$ ) as well as the experimental design (for example, number of replicates).

The participants grappled with what constitutes a biologically important difference between test and reference sediments. The discussion revolved around two issues. Most participants felt it was very important to characterize the variability of the sublethal response both in the presence and absence of reference sediment. For example, if the response normally varied by 20 percent, then a difference of at least that amount would have to occur before the results were considered biologically important (assuming statistical significance). The converse would also be true if the normal variability was small.

The second issue concerned the biological importance of the sublethal response itself. Reproduction and growth were identified as the most desirable sublethal endpoints. However, even these endpoints do not reflect potential impacts on the population—that level of biological organization which is most important ecologically and to society. The workshop participants could only identify one vector to link effects observed on individual organisms to population level impacts: demographic models.

Demographic models were originally developed to estimate probabilities, human mortalities, and future population patterns (Euler 1970 (originally published in 1760), Lotka 1925). Ecologists began using these models this century to examine life history characteristics of nonhuman species (Pearl and Miner 1935, Leslie 1945, and Ricker 1954). Marshall (1962) was the first to use demographic models in ecotoxicology. Very simply, these models integrate life history information (survival and reproduction) into population statistics such as the intrinsic rate of population increase or finite growth rate. Thus, the response of the individual becomes a population level response. The theory supporting these demographic models predicts population decline/extinction at levels defined *a priori*. For the most part, these models have not been field verified. In addition, the models make certain assumptions such as the absence of intraspecific competition and population steady-state, which are rarely met in nature. The participants felt that while demographic models represented an excellent way to express sublethal response to contaminated sediment, much more work was needed. Two areas specifically cited were verification of the models' predictive capability and improvement of experimental conditions under which the model parameters are generated.

## Workshop Summary

Important points made at workshop are listed below:

1. Presently no chronic sublethal sediment bioassays have been developed to a point where they can be used by the Corps of Engineers for the regulatory evaluation of dredged material.
2. A precise chronological definition for the term "chronic" is not possible. However, participants did agree that a chronic sediment exposure should include a substantial portion of the life cycle or number of life stages and allow sufficient time so that contaminant steady-state is approached in the tissues of the exposed animals.
3. The ability of an organism (or population of organisms) to reproduce and remain viable is of paramount ecological and societal importance. Desirable sublethal endpoints should strive to assess this capability.
4. Reproduction and growth were identified as the most desirable sublethal endpoints. Where possible, results should be expressed in terms of population level impacts.
5. The relationship between growth and reproduction must be established if growth is used as the sublethal endpoint.
6. Measures of growth should differentiate between somatic and gametic growth.

7. Prioritized criteria for species selection were developed and are:

- 1st - Intimate contact with sediment
- 2nd - Amenable to testing
- 3rd - Ecological relevance and sensitivity (tied)
- 4th - Economic importance

8. Amphipods and polychaetes were identified as the most desirable test species.

9. Sublethal sediment bioassay methods are most developed for the amphipod *Ampelisca abdita* and for the polychaete *Neanthes arenaceodentata*. Additional test development is still required for both species.

10. Other candidate species were identified but considerable test development is required.

11. Statistically significant results do not necessarily imply that biologically important differences exist between test sediments and reference sediments.

12. Additional work is required to fully develop interpretive guidance for chronic sublethal sediment bioassays.

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